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ARTYKUŁ SPECJALNY / *STATE-OF-THE-ART REVIEW*

Enjoy your heart-beets. The role of dietary inorganic nitrate in cardiovascular health

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INTRODUCTION

According to the World Health Organisation, since 2014, cardiovascular diseases (CVD) have been the primary cause of death, not only in developed countries, but also worldwide. Most CVD [1–3] and conditions such as post-ischaemic inflammation and no-reflow phenomenon [4, 5] are linked to disturbances in the function, structure, and integrity of arterial endothelium, collectively described as a pro-inflammatory and a pro-atherosclerotic phenotype of the endothelium.

A current view is that the diseased endothelial phenotype is a consequence of an increased vascular generation of reactive oxygen species (ROS) mediated by CVD risk factors, ROS-induced inactivation of the endothelium-derived nitric oxide (NO), and a resulting imbalance between the cellular signalling by NO and ROS [1–3, 6, 7]. Antioxidants failed to prevent CVD in clinical trials [8]. Instead, strategies intended to boost the NO signalling have emerged as a promising therapeutic objective for the prevention and treatment of CVD.

Vascular NO is generated though: (i) the classic L-arginine-NO synthase pathway and (ii) the newly described nitrate-nitrite-NO pathway in which the dietary inorganic nitrate (NO_3^-) (e.g. present in beetroot) undergoes *in vivo* conversion to nitrite (NO_2^-) and then to NO. Importantly, dietary NO_3^- and NO_2^- have been demonstrated to improve NO signalling and to induce beneficial cardiovascular effects [9–12]. Herein, current evidence is reviewed regarding: the role of NO and ROS in the mechanism of CVD, the role of NO_3^-/NO_2^- bio-activation in *in vivo* NO homeostasis, and the pro-health potential of the dietary NO_3^-/NO_2^-.

CLASSIC L-ARGININE/NO SYNTHASE PATHWAY

The major sources of NO *in vivo* are three NO-synthase (NOS) isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) isoform. The large mass of the endothelium causes eNOS to be the major NO producer. NOS uses NADPH, O_2, and tetrahydrobiopterin (BH_4) to convert L-arginine to L-citrulline with a concomitant release of NO (Fig. 1).

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$$\text{L-arginine} + O_2 + \text{NADPH} \xrightarrow[BH_4]{\text{NOS}} \text{L-citrulline} + \text{NO} + \text{NADP} + H_2O$$

Figure 1. Classic L-arginine/nitric oxide synthase pathway

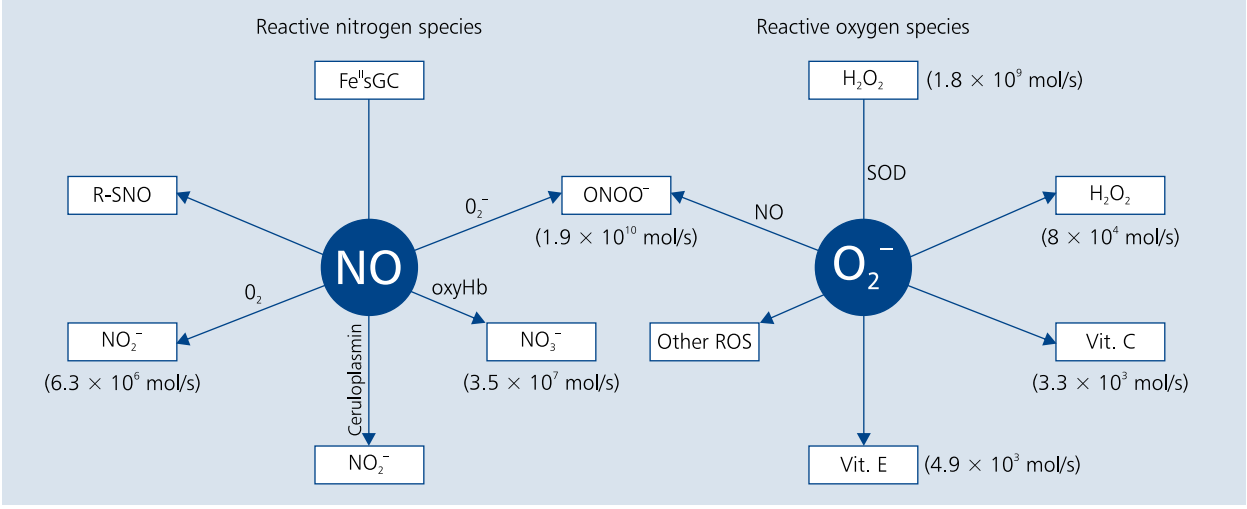

Figure 2. Major reactions of nitric oxide (NO) and superoxide (O_2^-) *in vivo*. The rate of some of these reactions is given in the parentheses. The O_2^- reaction with the antioxidant vitamins (Vit.) C and E is relatively slow (right-hand side), so these substances fail to compete effectively with the much faster reaction NO + O_2^- → ONOO$^-$ (centre). See text for more details. Fe^{II}sGC — Fe^{2+}-heme of soluble guanylyl cyclase; R-SNO — S-nitrosothiol; oxyHb — oxyhaemoglobin; SOD — superoxide dismutase; ROS — reactive oxygen species

However, under conditions of limited availability of L-arginine and/or BH_4, NOS activity switches from the NO to superoxide (O_2^-) generation (a process known as NOS uncoupling). Remarkably, the CVD risk factors are associated with eNOS uncoupling mediated by BH_4 deficiency (BH_4 undergoes peroxynitrite-mediated inactivation, see later) and/or increased production of a range of endogenous NOS false substrates, including asymmetric dimethyl arginine (ADMA) [13, 14].

The eNOS releases NO intra- and abluminally. It is expressed in the endothelium of large arteries, and its expression decreases in small resistance arteries and veins and is virtually absent in the capillaries [15]. The laminar blood flow and the related endothelial shear stress are the major eNOS activators. Short-lasting and long-lasting increases in the shear stress (e.g. accompanying physical exertion) stimulate the eNOS activity and the expression, respectively. Also, erythrocytes express eNOS and produce NO, although the significance of these phenomena remains unclear [16].

Nitric oxide is a signalling molecule preferentially targeting heme proteins and protein cysteine residues (Fig. 2, left-hand side). Binding of NO to the Fe^{2+}-heme of soluble guanylyl cyclase activates this enzyme to produce cyclic guanosine 3,5-monophosphate (cGMP). cGMP in turn activates protein kinase G, which mediates phosphorylation of proteins in vascular smooth muscle (phosphatases, ion channels) (Fig. 3A), resulting in vascular relaxation. The *in vivo* half-life of NO is < 1 s; therefore, its cGMP-dependent effects (elicited in an autocrine/paracrine manner) are rather local. However, NO exerts most of its effect via two more stable mediators S-nitrosothiols and NO_2^- (the latter being a physiological store of NO, see later) that act in an endocrine- and cGMP-independent manner. S-nitrosothiols are products of the reaction of protein S-nitrosylation, which involves addition of the nitrosyl (–N=O) moiety to the cysteine thiol (–SH group) side chain of a protein (Fig. 3B). The nitrosyl group can then be exchanged between cysteine thiols of the same or neighbouring proteins. The process of transnitrosylation, has the effect that circulating S-nitrosothiols may accumulate in the peripheral tissues, and that they are relatively stable, which explains the wide range of cellular effects of the endothelial NO within and outside the cardiovascular system. The S-nitrosylation results in protein posttranslational modifications and eventually may have an effect on gene expression. The activation of endothelial cells has been reported to mediate the S-nitrosylation of > 100 proteins, including mitogen-activated protein kinases, thyrosine kinases, phosphatases, transcription factors, innate immune system receptors, and β_2-adrenergic receptors [17].

The majority of the endothelium-derived NO is rapidly inactivated to biologically inactive NO_3^- by haemoglobin and myoglobin present in erythrocytes and muscle cells, re-

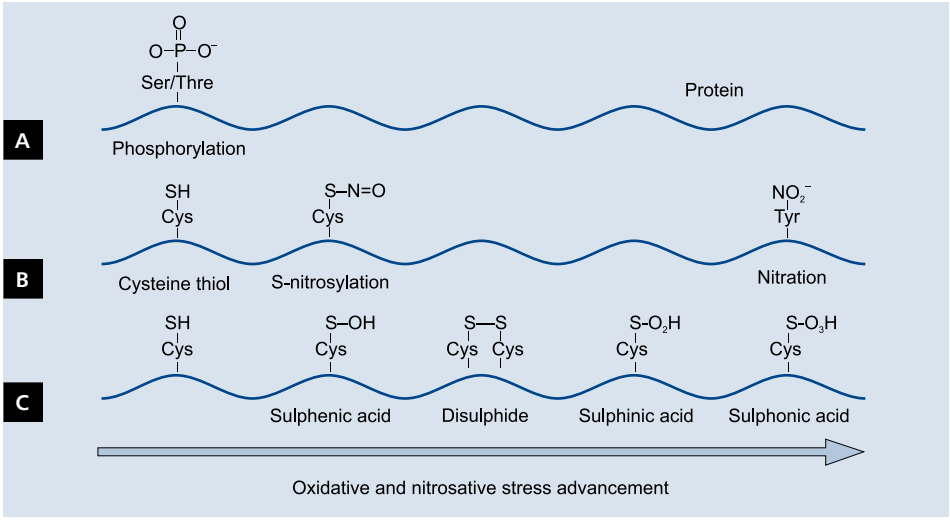

Figure 3. Mechanisms of post-translational protein modifications by reactive nitrogen species (RNS)- and reactive oxygen species (ROS). Nitric oxide (NO) acts via (**A**) cyclic guanosine 3,5-monophosphate (cGMP) and protein kinase G-dependent phosphorylation of protein serine/threonine (Ser/Thre) residues; (**B**) cGMP-independent S-nitrosylation of protein cysteine (Cys) thiols (which may also be mediated by NO_2^-) and (**B**, right) peroxynitrite-dependent irreversible nitration of protein tyrosine (Tyr) residues. Also ROS-mediated signalisation (**C**) occurs via protein cysteine thiol modifications progressing from sulphenic acid, via disulphide and sulphinic acid, up to irreversible sulphonic acid

spectively. However, a small fraction of the NO is converted to a biologically active NO_2^- either by ceruloplasmin [18] or via NO autoxidation (Fig. 2). Thereby, serum NO_3^- levels are ~100 times higher than NO_2^- in healthy subjects (~30 μM vs. 150–300 nM) [19].

The biologically favoured NO reaction is that with O_2^- ($NO + O_2^- \rightarrow ONOO^-$) (Fig. 2). Consequently, the reported effects of vascular O_2^- overproduction (as associated with the CVD risk-factors) included: (i) impaired vascular NO bioavailability (seen as the endothelial dysfunction in the FMD test); (ii) vascular overproduction of the toxic peroxynitrite ($ONOO^-$) mediating BH_4 inactivation, the eNOS uncoupling and further impairment of the NO availability, and (iii) decreased S-nitrosothiol and NO_2^- production and impairment of their signalisation [15]. Peroxynitrite may cause nitration of protein tyrosine residues to form 3-nitrotyrosine (Fig. 3B). While protein phosphorylation and S-nitrosylation are part of the normal cellular regulatory mechanism, protein nitration is an irreversible toxic process. Actually, plasma and/or tissue 3-nitrotyrosine is a biomarker of the nitrosative stress.

O_2^- AND THE REACTIVE OXYGEN SPECIES PATHWAY

A major source of the vascular O_2^- is NADPH oxidase (Nox), particularly its isoforms Nox1 and Nox2, expressed in the endothelial and vascular smooth muscle cells. Nox1/2 constitutively generate O_2^-, which in turn stimulates O_2^- generation by other enzymatic systems (mitochondria, xanthine oxidase, and eNOS) [3, 20, 21]. Once generated, O_2^- initiates a cascade of oxidative reactions causing the generation of other ROS (Fig. 2, right-hand side). The reaction: $O_2^- + NO \rightarrow ONOO^-$ is biologically favoured. Much slower are two O_2^- dismutation reactions (one catalysed by the superoxide dismutase [SOD] and the other spontaneous) yielding hydrogen peroxide (H_2O_2), which in turn may become a source of a toxic hydroxyl radical.

The major mechanism of the cellular signalisation by O_2^- and H_2O_2 involves oxidative modification of protein cysteine thiols (Fig. 3C). Thus, nitrosative (Fig. 3B) and oxidative modifications of cysteine thiols compete with each other. With the increase in ROS availability, cysteine thiols undergo the modifications progressing from sulphenic acid (S-OH) via disulphide (S-S) and sulphinic acid ($S\text{-}O_2H$) up to irreversible sulphonic acid ($S\text{-}O_3H$). Disulphide formation can be internal, or mixed between proteins. These oxidative modifications represent a graded transition from normal signalling functions, through adaptation to oxidative stress (e.g. S-S), and finally to toxicity (sulphonic acid) [22, 23].

NITRIC OXIDE AND ROS INTERACTIONS VS. THE PRO-ATHEROSCLEROTIC ENDOTHELIAL PHENOTYPE

The endothelium maintains vascular and systemic homeostasis through multiple interactions with cells within the vessel wall and the blood (Table 1). These interactions are mediated mainly by endothelium-derived autacoids such as NO and O_2^-, but also by prostaglandins, endothelin-1, and others. The endothelial homeostasis is disrupted in CVD and states such as ischaemia-reperfusion, and the resulting changes in the endothelial phenotype can be identified as:

Table 1. Arterial endothelial phenotype in health and cardiovascular diseases (CVD)

Health	Endothelial feature and/or endothelium-mediated activity	CVD
Tight	Barrier separating blood and sub-endothelial tissue	Leaky
↓	Vascular tone	↑
↓	Platelet activity	↑
↓	Clotting cascade activity	↑
↑	Fibrinolytic activity	↓
↓	Adhesion molecules expression	↑
↓	Pro-inflammatory cytokine production	↑
↓	Leukocyte adhesion, activation, and trafficking	↑
↓	Vascular smooth muscle cell and media growth	↑
↑	Re-endothelialisation and endothelial healing	↓
↑	Angiogenesis	↓

↑ — activation; ↓ — inhibition

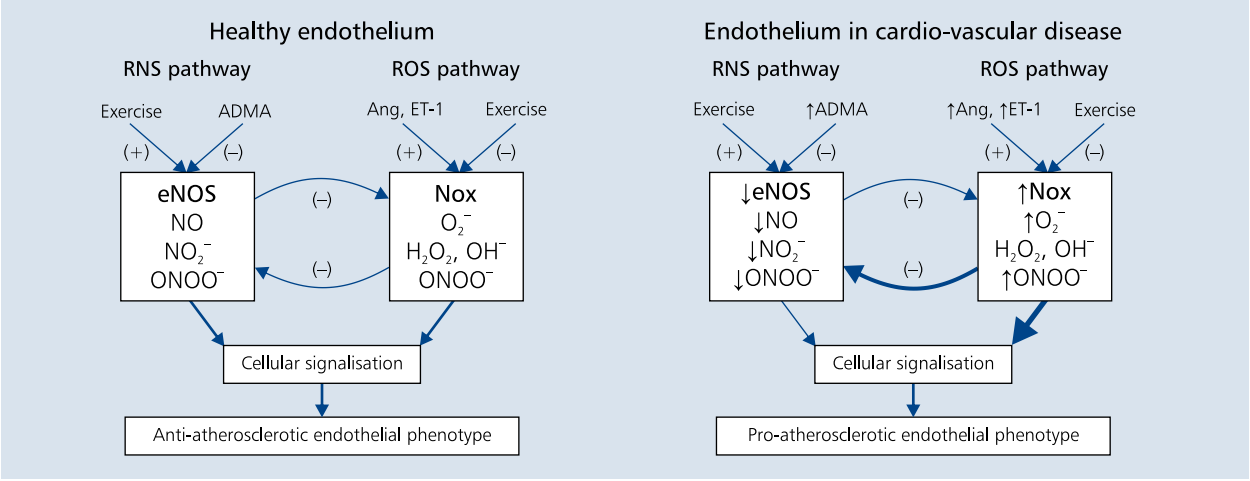

Figure 4. Vascular endothelium phenotype as determined by the balance between reactive nitrogen species (RNS)- and reactive oxygen species (ROS)-mediated cellular signalisation. The balance is preserved (left) in healthy endothelium, resulting in its anti-atherosclerotic phenotype. The balance is disrupted (right) by the cardiovascular disease risk factors, which, via several mechanisms, boost ROS pathway and downregulate RNS pathway, altogether mediating the development of the pro-atherosclerotic endothelial phenotype (see text for more details)

(i) an impairment of endothelium-dependent vasodilatation; (ii) a pro-inflammatory state; (iii) a pro-thrombotic state, and (iv) a state promoting arterial wall proliferation [15, 24].

The endothelial phenotype is currently viewed as a product of the competition between endothelial signalling mediated predominantly by NO and its reactive metabolites (reactive nitrogen species [RNS]) and by O_2^- and its reactive metabolites (reactive oxygen species [ROS]). An emerging paradigm is that (Fig. 4):

1. Even healthy vessels produce some basal amounts of NO and O_2^- mediating the physiological endothelial signalling functions.
2. A major source of the vascular NO and O_2^- are eNOS and Nox. Major inducers of the activity and the expression of eNOS and Nox1/Nox2 are exercise and agonists such as angiotensin II and endothelin-1, respectively. In addition, RNS and ROS pathways mutually inhibit their activity and expression.
3. While RNS pathway dominates in the healthy endothelium, the diseased endothelial phenotype is a consequence of an imbalance between the endothelial signalling by RNS and ROS caused by the vascular ROS overproduction [1–3, 6, 7].

The CVD risk factors (hypercholesterolaemia, hypertension, diabetes, and others) acting mostly via angiotensin II and endothelin-1 [3, 7], and ischaemia/reperfusion acting via endothelin-1 [4, 5], all impair the endothelial NO bioavailability via four potential mechanisms: (i) the agonists mediated up-regulation of the vascular Nox1/Nox2 expression and increased production of an excess vascular O_2^-, which

in turn inactivates NO to form $ONOO^-$ [1–3, 6, 7]; (ii) oxidative stress-mediated downregulation of the eNOS expression; (iii) oxidative stress-mediated eNOS inhibition related to the up-regulated production of the endogenous eNOS inhibitors (e.g. ADMA) [14]; and (iv) eNOS uncoupling caused by ADMA and/or $ONOO^-$-induced BH_4 inactivation [13].

Exercise training is a major natural activator of eNOS, an inhibitor of Nox, an inductor of anti-atherosclerotic endothelial phenotype, and hence an effective protective measure against CVD [25]. This is probably related to the fact that exercise, by increasing arterial laminar blood flow velocity and endothelial shear stress, up-regulates eNOS and endothelial NO production, which in turn down-regulates vascular Nox and vascular O_2^- generation [2, 25, 26]. In addition, exercise training was shown to normalise, in an endothelium-dependent manner, increased sympathetic nervous system activity and increased renin–angiotensin system activity in patients with CVD [25]. Actually, a prolonged pharmacological inhibition of eNOS resulted in increased vascular expression of Nox1/Nox2 and in oxidative stress in experimental models, altogether supporting the concept that the endothelial ROS and RNS pathways control each other (Fig. 4).

Currently, the endothelial phenotype can only be indirectly clinically assessed. One approach is to evaluate the endothelial NO-availability by assessing the flow-induced and NO-dependent vasodilation (using the FMD test, for instance). It appears that the impairment of such a response, dubbed "endothelial dysfunction", correlates with the presence and the progression of various forms of CVD, and that the measures preventing CVD also reduce the endothelial dysfunction [24, 27], supporting the view that disrupted endothelial homeostasis underlies the mechanism of CVD.

THE NO_3^--NO_2^--NO PATHWAY

Recently, it has become clear that: (i) inorganic NO_2^- is a substrate for endogenous NO_2^--reductases and their *in vivo* production of authentic NO; (ii) ~70% of NO_2^- present in the blood and/or stored in tissues is derived from the L-arginine/NOS pathway and the remaining 30% is acquired through dietary intake; (iii) NO_3^- and NO_2^- ingestion increases plasma levels of NO_3^-/NO_2^- in humans, and a diet depleted of the NO_3^-/NO_2^- decreases these levels (at least in animals); (iv) estimates of NO_3^- intake from food are 93–124 mg/day in Europe (60–80% of this from vegetables and the reminder from drinking water) and as much as ~1100 mg/day in Japan [28]; and (v) the NO_3^- component of vegetables contributes to the beneficial health effects of this food group, including protection against CVD [10, 11, 23, 29].

The ingested NO_3^- is absorbed in the upper gastrointestinal tract and reaches peak plasma concentration 30–60 min after ingestion. Within a 24-h period ~75% of the absorbed NO_3^- is excreted by the kidneys. The remaining ~25% is taken up by the salivary glands and then gradually secreted with the saliva into the oral cavity, where commensal bacteria on the tongue convert ~20% of the NO_3^- present in the saliva (i.e. ~5% of the originally ingested NO_3^-) to NO_2^- [10, 11, 23]. The NO_2^- is swallowed, and its proportion is protonated in the stomach, forming nitrous acid, which in turn decomposes to NO, which ensures the normal gastric mucosa physiology and serves the first-line host defence against pathogens [29]. The NO_2^- that escaped protonation in the stomach enters the systemic circulation (reaching the peak plasma NO_2^- concentration 2–3 h after NO_3^- ingestion) and is partially stored in the peripheral organs, enabling its local and/or systemic (endocrine) activity [10, 11, 23]. The biological activity of the NO_2^- is related to the fact that it, *per se*, induces protein S-nitrosylation [30] and/or undergoes enzymatic reduction to NO (Fig. 5) [10, 11, 23, 29].

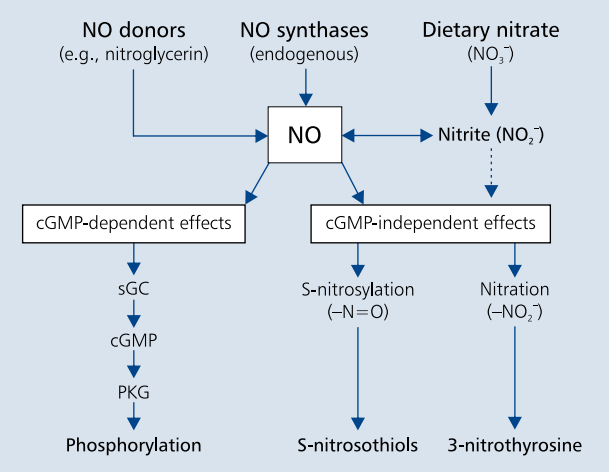

Figure 5. *In vivo* nitric oxide (NO) metabolism. NO may originate from: (i) drugs; (ii) NO synthases generating NO from L-arginine; and (iii) dietary inorganic NO_3^- that, via NO_2^-, is converted to NO. NO is oxidised to NO_3^- (not shown) and NO_2^-. NO-mediated post-translational protein modifications and/or changes in genes expression occur via protein phosphorylation, S-nitrosylation, and nitration. NO_2^- exerts its effects via NO and/or the NO_2^--induced protein S-nitrosylation; sGC — soluble guanylyl cyclase; PKG — protein kinase G; cGMP — cyclic guanosine 3,5-monophosphate

Nitrite is reduced to bioactive NO by deoxyhaemoglobin (in erythrocytes], deoxymyoglobin (in cardiomyocytes and skeletal muscle cells], xanthine oxidase (in erythrocytes and endothelial cells], and some mitochondrial enzymes [10]. Importantly, the effectiveness of these systems to reduce NO_2^- to NO increases in acidic conditions, such as those associated with tissue ischaemia/hypoxia. In contrast, ischaemia/hypoxia impair the NO formation by NOS [10].

THERAPEUTIC UTILITY OF THE NO_3^--NO_2^--NO PATHWAY

A number of epidemiological studies have shown that fruit- and vegetable-rich diets (e.g. DASH diet, traditional

Table 2. Nitrate content of food products. Modified from [23]

Food product	NO_3^- concentration [mg/100g]	
	Mean	Range
Beets	275.6	168–359
Spinach	233.3	53.5–366
Radishes	168.0	76.4–250
Celery	154.4	31.6–332
Lettuce	85.0	7.9–217.1
Iceberg lettuce	78.6	34.7–108
Mushroom	59.0	1.9–8.5
Cabbage	57.3	19.3–97.6
Broccoli	39.4	2.9–114
Green beans	38.6	16.5–61.1
Strawberries	17.3	10.5–29.3
Banana	13.7	8.8–21.1
Green pepper	3.3	0.8–5.5

Mediterranean and Japanese diets) protect against CVD, type 2 diabetes, stroke, and conditions such as osteoporosis and cancer [9, 23, 31]. These effects have been attributed to flavonoids contained in these foods. However, this interpretation is complicated by the fact that vegetables and fruits are rich in salutary inorganic NO_3^- (Table 2) [28, 31]. Some of the beneficial effects of NO_3^- in the cardiovascular system are reviewed below.

Blood pressure and arterial stiffness

Dietary supplementation of NO_3^- and NO_2^- has been reported to reduce blood pressure in hypertensive rats [32] and in normotensive and hypertensive subjects [10, 11, 23]. These effects of NO_3^- were dose-dependent, and similar effects were exerted by NO_3^- given in the form of beetroot juice and KNO_3^- [33]. For instance, a single ingestion of 500 mL of a beetroot juice (1395 mg NO_3^-) caused the following in normotensive subjects: an increase in serum NO_2^- levels and a corresponding 24-h reduction in the systolic and diastolic blood pressure (by ~10 mm Hg and 8 mm Hg, respectively), improvement in the endothelium-dependent vasodilatation, and platelet aggregation inhibition. These effects were absent if the subjects refrained from swallowing their saliva, therefore interrupting entero-salivary circulation and preventing the rises in plasma NO_2^- levels [10]. The importance of this circulation was further supported by the fact that antibacterial mouthwash resulted in the reduction in NO_2^- plasma levels and caused a concomitant increase in blood pressure in normotensive subjects, again confirming the regulatory role of NO_2^- [34]. Interestingly, it has been reported that a single dose of beetroot juice (205 mg NO_3^-) exerted a much stronger hypotensive effect in hypertensive than in normotensive subjects. In addition, the expression of the erythrocyte xanthine oxidase was greater in hypertensive than in normotensive subjects, and the hypotension by NO_3^- was effectively blocked by a xanthine oxidase inhibitor, allopurinol [11, 32]. Altogether, these data suggested that it was xanthine oxidase that mediated NO_2^- conversion to NO, and the NO_3^--mediated hypotension [32]. Furthermore, a recent randomised, placebo-controlled study in drug-naïve hypertensive patients (18–85 years) demonstrated that also prolonged NO_3^- supplementation (250 mL/day of beetroot juice for four weeks) caused blood pressure reduction, which was sustained and was associated with improved endothelium-dependent vasodilation and a reduction in arterial stiffness [35]. Also, NO_2^- was found to exert beneficial vascular effects. For instance, age-dependent: (i) vascular oxidative stress; (ii) reduction in NO_2^- plasma levels; (iii) impairment of endothelium-dependent vasodilatation, and (iv) a rise in arterial stiffness were all prevented by NO_2^- (50 mg/L in drinking water) in mice [36]. Likewise, NO_3^- (9.3 mg/kg/d. for four weeks) was demonstrated to decrease pulse wave velocity in a randomised, placebo-controlled study in healthy, aged volunteers (65 ± 5 years) [37]. Overall, these findings suggest the efficacy of NO_3^-/NO_2^- in the therapy of the hypertension and the prevention of age-associated CVD.

NO_3^-/NO_2^- and ischaemia/reperfusion injury

Several studies have demonstrated efficacy of dietary NO_3^-/NO_2^- in ameliorating myocardial ischaemia/reperfusion injury and/or in reducing infarct size in animal models [10, 11, 23]. Conversely, cardiac and hepatic ischaemia/reperfusion injury were increased and serum and tissue levels of NO_2^- were decreased in animals fed with NO_3^-/NO_2 depleted food [30]. Overall, these findings indicated NO or NO_2^- as inducers of the protection, and became a basis for further clinical studies. In one such study, patients with ST elevation myocardial infarction were given $NaNO_2$ (70 μmol IV) 5 min before the reperfusion, and no beneficial effects on infarct size and long-term prognosis were observed [38]. However, it is conceivable that NO_2^- takes a much longer time to develop its action. The results of a similar study in which NO_2^- was given directly to the coronary are awaiting publication [39]. Likewise, a randomised, double-blind, placebo-controlled trial testing the effect of $NaNO_3$ pretreatment (700 mg on the eve of the coronary artery bypass grafting and then 3 h prior to the surgery) on surgery-induced myocardial injury is also awaiting publication (clinicaltrials.gov: NCT01348971).

NO_3^-/NO_2^- vs. exercise tolerance

Several studies in healthy untrained and trained subjects revealed that dietary NO_3^- supplementation ($NaNO_3^-$ or beetroot juice) dose-dependently increased exercise capacity and simultaneously decreased the oxygen cost of the exercise [11, 40, 41]. This latter effect is probably attributable to the fact that enzymes of the mitochondrial respiratory chain act as NO_2^- reductases to produce NO [10], and that NO favour-

ably modifies the mitochondrial oxygen cost of adenosine-5'--triphosphate formation [11]. In this context, it has been shown that dietary NO_3^- supplementation resulted in a 19% improvement in the efficiency of mitochondria (P/O ratio) isolated from skeletal muscles of healthy volunteers [42]. Likewise, a single dose of NO_3^- (558 mg) was reported to increase by ~20% the walking distance in patients with peripheral arterial disease [43]. However, it is unclear if this effect was due to improved energetics or some anti-ischaemic effect. Also, the long-term effectiveness of the NO_3^-/NO_2^- treatment needs to be investigated.

NO_3^-/NO_2^- vs. insulin resistance

Impaired NO availability (seen as endothelial dysfunction), such as that usually accompanying the CVD risk factors, is always associated with insulin resistance. Interventions improving endothelial function were shown to reduce insulin resistance and, *vice versa*, interventions improving tissue insulin sensitivity improve also the endothelial function [44]. These data suggested the existence of a cause-effect relationship between the disturbances in the NO, insulin, and glucose metabolism, a view supported by experimental studies [23, 45]. For instance, insulin has been shown to stimulate endothelial NO generation and skeletal muscle blood flow, which in turn is a major determinant of insulin-dependent glucose uptake in skeletal muscles [44]. On the other hand, mice with the eNOS gene knockout were shown to develop a metabolic syndrome-like phenotype, which could be prevented by dietary NO_3^- supplementation [46]. It has also been demonstrated that, at least in rats, NO_3^- and NO_2^- increase pancreatic insulin secretion [47], which altogether suggests that NO and insulin mutually stimulate their generation. Nevertheless, the only study aimed at clinical verification of these encouraging experimental observations provided disappointing results. In this randomised, double-blind study, patients with type II diabetes drank beetroot juice 250 mL/day for two weeks, which had no effect on their blood pressure, endothelium-dependent vasodilatation, or insulin resistance [48].

PHARMACOKINETICS AND TOXICOLOGY OF NO_3^-/NO_2

The salutary effects of NO_3^-/NO_2 seen in experimental and small clinical studies might eventually translate into their use as pharmaceutical agents. The practical aspects of this would be as follows.

1. NO_3^- and NO_2^- are cheap and easily applicable (diet supplemented with vegetables and/or inorganic NO_3^-/NO_2^-), and an equipotent hypotensive effect of beetroot juice and $NaNO_3$ was reported [10].
2. In contrast to organic nitrates (e.g. nitroglycerin) [49], inorganic NO_3^-/NO_2 does not result in the nitrate-tolerance phenomenon. Furthermore, NO_3^-/NO_2 prevents, but does not induce, vascular oxidative stress [11, 37].
3. Although the therapeutic potential of NO_3^- and NO_2^- is similar, a therapeutic profile of NO_3^- seems to be more favourable. Thus, inorganic and dietary NO_3^- have a much longer half-life in human plasma (~6 h) compared to NO_2^- given either by oral or IV routes (15–45 min), meaning that NO_3^-, in contrast to NO_2^-, could be given as a once-daily dosing regimen [11].
4. The L-arginine/NOS pathway is oxygen-dependent. The NO_2^- conversion to NO increases with increasing acidosis and hypoxia. Therefore, manipulations of the latter pathway may be particularly suited to conditions with accompanied organ ischaemia/hypoxia.
5. In the 1970s a debate was initiated as to the safety of the ingested NO_3^- or NO_2^-, particularly those used in the process of cured meat preparation. It was argued that NO_3^- and NO_2^- may become a source of carcinogenic N-nitrosamines in food, and the use of NO_3^-/NO_2 for curing was almost banned. However, according to recent epidemiological studies, there is no evidence for the carcinogenicity of NO_3^-/NO_2 in food, meaning that current recommendations as to the permissible concentration of NO_3^-/NO_2^- in food and water may be too restrictive [11, 28]. Instead, emerging evidence suggests therapeutic utility of NO_3^- and NO_2, a prospect awaiting verification in large-scale clinical trials.

Conflict of interest: *none declared*

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